

The University of Chicago

THE TESTICULAR HORMONE CON-
TENT OF HUMAN URINE

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
AND PHARMACOLOGY

By
ENNIS BRYAN WOMACK

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THE TESTICULAR HORMONE CONTENT OF HUMAN URINE*

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Loewe, Voss, Lange and Wöhner (1) reported that by their seminal vesicle "regeneration test" on the castrated mouse, they found it necessary to use the equivalent of 3.6 liters of male urine to demonstrate the presence of the male hormone therein. Funk, Harrow and co-workers (2, 3, 4), as well as workers in this laboratory, showed that by the comb growth method testicular hormone in the urine of adult men can easily be detected by injecting the equivalent of 50 to 150 cc. of urine per capon per day. The earlier investigations (5, 6, 7, 8) in these laboratories on the extraction of the testis hormone from testis tissue naturally led us to investigate other tissues and fluids. In this paper we present some of our recent findings on human urine.

A. METHODS

We first investigated various methods for the extraction of the comb growth stimulating hormone from urine with the view of devising a comparative quantitative procedure applicable to a 24-hour sample of urine. Among the various attempts may be mentioned: (a) continuous ether extraction; (b) continuous ether extraction of the acidified urine followed by fractionation in cold acetone, resolution in ether and shaking with 10 per cent NaOH; (c) butyl alcohol extraction; (d) refluxing of urine with ether on a water bath; (e) shaking urine or concentrated urine with ether on a water bath; (f) shaking urine or concentration urine with equal volumes of benzene, ether, or chloroform; and (g) continuous extraction of acidified urine with benzene for 24 to 36 hours. The best and most consistent results have been obtained by extracting acidified urine by benzene. This method of extraction together with the treatment by 10 per cent NaOH gave the most consistent results with least toxic action on the birds.

The procedure finally adopted for these studies is the following: The urine is evaporated to one-fifth its original volume, then after adding 20 cc. of concentrated hydrochloric acid per liter of original urine, it is shaken at room temperature with an equal volume of benzene. This first benzene extract is set aside. The urine layer is then placed in a Kutscher continuous extractor and extracted for 30 to 36 hours with fresh benzene. The two benzene extracts are combined and evaporated to dryness under diminished pressure, care being taken to remove the benzene as completely as possible. The residue, after dissolving in 100 cc. of ether per liter of

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original urine, is vigorously shaken with one-tenth the volume of 10 per cent NaOH. The aqueous layer is repeatedly shaken with fresh ether and the combined ether extracts evaporated under diminished pressure and taken up in the proper volume of olive oil.

For the assays we used Brown Leghorn capons which had been castrated at the age of six to eight weeks by Dr. L. V. Domm of the Whitman Laboratory of Experimental Zoology. We wish to thank Dr. Domm for this assistance. The birds were kept in large pens at the Whitman Laboratory with free access to the outdoors. The olive oil solutions of the extracts were injected subcutaneously daily for periods of five to eight days and the comb growth is recorded as the sum of the increases in length and height.

B. THE EFFECT OF AGE ON THE TESTICULAR HORMONE EXCRETION

Funk, Harrow and Lejwa (3) reported a few results which indicated that the urine from men in the prime of life contains more hormone than from old men. Our results on the hormone in testes at different ages suggested an investigation of a similar character on the relation of age to hormone excretion. We have not studied the urines of old men, but have emphasized those of children and men in the prime of life. (See Table I.)

TABLE I
ASSAYS ON MALE URINE AT DIFFERENT AGES

Fraction	Age	Source	Quantity Extracted	Daily Dosage Equivalent to Urine cc.	Bird No.	mm. Comb Growth
62a	7 years....	R. G.....	24 hr. S.....	72	22	0
62b	7 years....	R. G.....	24 hr. S.....	70	987	0
69a	3 years....	M. H.....	24 hr. S.....	75	7	0
69b	3 years....	M. H.....	24 hr. S.....	80	11	0
K-83	4-10.....	Composite...	6 liters.....	600	966	0
K-84a	14-20.....	High Sch....	8 liters.....	270	20	12
K-84b	14-20.....	High Sch....	8 liters.....	270	44	10
K-65	Adult.....	Composite...	3400 cc.....	340	57	12
K-66	Adult.....	Composite...	3500 cc.....	350	955	15
K-70-B1	Adult.....	Composite...	4 liters.....	400	17	14
K-73	Adult.....	24 hr. S.....	1300 cc.....	130	57	9
K-90	Adult.....	24 hr. S.....	1000 cc.....	100	953	8
K-53	Adult.....	Composite...	800 cc.....	80	14	6
K- 3	Adult.....	24 hr. S.....	850 cc.....	85	514	8

The extracts from individual and composite samples of urine from boys under ten years of age uniformly gave negative results. The daily injections with the individual 24-hour samples represented only 70 to 80 cc. of urine, but since the total volume of the 24-hour sample was only 500 to 600 cc., this dosage is equivalent to the injections employed of the adult urine. Furthermore, since the injection of the equivalent of 600 cc. urine daily of the composite samples gave equally negative results, these experiments seem to warrant the conclusion that the hormone concentration must be very much lower than that found in urine of men where the equivalent of 50 to 150 cc. per day is ample to give a good response in comb growth. The urines from boys 14 to 20 years of age gave values comparable to those of mature men.

The negative results from urine of boys under ten years of age may appear contradictory to our findings on the testes from foetal and two months' old calves. However, when the equivalent of 400 cc. of bull calf urine per day was injected over a period of eight days, it gave negative results. Therefore, we are inclined to think that although the immature testes contain the hormone, they are either not secreting the same or if the hormone is secreted, it must be destroyed more completely or conserved more economically as a result of a different kidney threshold than in the normal adult male. The fact that evidences of secondary sex character become evident more particularly at puberty is a physiological implication that the testes in the foetal and prepubertal stages are not secreting the hormone to the same extent as in the adult.

C. EXTRACTION OF A COMB GROWTH STIMULATING SUBSTANCE FROM WOMEN'S URINE

Laqueur and his co-workers (9) as well as others have reported the presence of the female sex hormone in urine from normal young men. Very recently Brouha and Simonnet (10) reported that pregnancy urine up to two months' term, when injected into normal immature male rats, causes a more rapid development of the genital tract whereas in normal adult male rats it causes hypertrophy thereof. This effect they attribute to the action of the anterior pituitary hormone. As no reports have been made on female urine, we undertook to examine urine from ovariectomized, normal mature and pregnant women.

The same method of extraction as previously described was used in all of these studies. The results are given in Table II. The values obtained show that normal and pregnancy urines from adult women yield the same amount of comb growth stimulating material as urine from normal mature men. The one case of the ovariectomized woman gave two negative results, although the equivalent of 400 cc. of urine was injected. Pregnancy urine

TABLE II
ASSAYS ON FEMALE URINE EXTRACTS

Sample	Source	Bird No.	Daily Dosage Equivalent to Urine cc.	mm. Comb Growth	Vaginal Smear Test
K-60	Ovariectomized.....	28	400	0	
K-62	Ovariectomized.....	954	400	0	
*K-63	Pregnancy.....	2	250	14	
K-61	Normal.....	995	500	18	
K-67	Normal.....	4	185	7	Negative
K-68	Normal.....	954	180	13	Negative
K-69	Normal.....	6	250	12	
K-71	Normal.....	996	220	9	Negative
K-72	Normal.....	46	245	11	Negative
K-74	Normal.....	40	98	7	
K-75	Normal.....	47	130	0	
K-76	Normal.....	45	175	5	Negative
K-77	Normal.....	974	245	13	
K-78	Normal.....	4	270	8	

*A composite sample which also gave a positive assay for female hormone according to test developed by Juhn and Gustavson.

gives an average result slightly higher than non-pregnancy urine. More studies must, however, be made before we can state this to be the general rule. The pregnancy sample here used was part of a composite sample, while the normal non-pregnancy samples were all 24-hour collections. It is worth noting that although our extract from the pregnancy sample gave the typical female feathering response as observed by Juhn and Gustavson and therefore contained female sex hormone, we nevertheless obtained almost equally good comb growth from normal female urine preparations which gave negative vaginal smear tests in the hands of Professor R. G. Gustavson and Dr. Fred E. D'Amour. In none of the other cases did we observe the female feathering after the injection of our extracts. We are inclined to interpret these comb growth reactions as due to the male hormone or to a substance possessing similar solubilities and physiological actions. That it is not the female hormone which causes this comb growth response is indicated, first by the negative vaginal smear reactions above, second by the negative comb growth response from purified theelin preparations and third, by the following personal communication from Professor R. G. Gustavson. In the course of the work reported on by Juhn, Gustavson and Faulkner (11, 12) they frequently observed comb growth as well as female feathering when the capons were injected with crude female hormone preparations from pregnancy urine while at other times the comb growth response was negative, although the female feathering response remained.

After we obtained the results here indicated, Professor Gustavson examined his protocols and found that the positive comb response was obtained only in those cases in which the purification by removal of the female sex hormone into 10 per cent NaOH was not carried out as completely as in those cases in which feather changes and positive vaginal smear tests were obtained without comb growth response. Thus, capon 29-939, which received a daily injection of 9 rat units (vaginal smear basis) per 100 grams body weight of a crude preparation from pregnancy urine from December 4 to December 19, showed an increase in comb growth of 19 mm. (L+H), while capon 29-984, with a daily injection of 400 rat units (the equivalent of 1200 cc. of pregnancy urine) of a highly purified theelin preparation, showed marked feather changes but an actual decrease in the size of the comb. We feel that the ether-10 per cent NaOH treatment, if properly carried out, actually separates from each other the female and the male hormones here under discussion. That the action is not due to anterior lobe secretion is also demonstrated by the negative comb growth response observed after two capons received daily for ten days, five times the dose of the gonad-stimulating preparations necessary to cause precocious sexual development in the immature white rat. We are indebted to Mrs. Zonja Wallen-Lawrence and Professor H. B. van Dyke of our Pharmacological Laboratory for this preparation.

We feel that our results warrant the conclusion that we have present in urine from pregnant and normal mature women, a substance very sim-

ilar to or identical with the testicular hormone, but that it is different chemically in some respects and acts differently physiologically from the female sex hormone which produces oestrus in the castrated female. The actual source and function of this hormone is at present a mystery. Although the urine from the ovariectomized woman gave negative tests, we do not regard this one case as sufficient evidence to be considered in this connection. On the other hand, Gallagher (13) in these laboratories could not find the testicular hormone in cattle ovaries, although he made three attempts.

SUMMARY

1. A method for the comparative quantitative extraction of 24-hour collections of human urine was devised.

2. No comb growth stimulating hormone was found in urine from boys under ten years of age.

3. The urine of adolescent boys yielded quantities of hormone of the same order as the urine from mature men.

4. The urine of pregnant and normal women yielded quantities of the hormone of the same order as the urine from mature men.

5. The comb growth response from pregnancy urine could not be duplicated by the action of theelin.

6. It was found that the comb growth stimulating hormone and the theelin in women's urine can be separated from each other by the ether-10 per cent NaOH treatment.

7. The quantitative aspects of the work here reported are necessarily incomplete due to the limited number of birds available and also due to the limited number of assays that can be made from a 24-hour collection of urine.

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